Docket No.: 2870-0330PUS1
(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| Patent Application of: | Atsushi MURAGUCHI et al. | | |
| Application No.: | 10/573,289 | Confirmation No.: | 9388 |
| Filed: | February 20, 2007 | Art Unit: | 1797 |
| For: | MICROWELL ARRAY CHIP AND ITS MANUFACTURING METHOD | Examiner: | M. L. Hobbs |

**DECLARATION UNDER 37 C.F.R. § 1.132**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Tsutomu Obata, declare as follows:

1. I am a co-inventor of the present application.

2. I received my doctorate in engineering from the University of Toyama, Faculty of Engineering, in March, 1997.

3. I am employed as a Researcher at the Toyama Industrial Technology Center Central Research Institute. My research involves the development of sensors and bio-chips using Micro Electro Mechanical Systems (MEMS).

4. My employment history is as follows:

2004-present Toyama Industrial Technology Center Central Research Institute, Researcher

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| 2002 | Japan Society for the Promotion of Science, (JSPS), Domestic Research Fellow |
| 2001 | Japan Science and Technology Corporation, (JST), Domestic Research Fellow |
| 1997 | Hokuriku Electric Industry Co., Ltd.<br>Development Department Group Leader, (MEMS)<br>Tohoku University Division of Mechanical Engineering, Esashi Lab, Researcher |
| 1992 | Hokuriku Electric Industry Co., Ltd. Central Research Institute |

5. The claimed invention is directed to a microwell array chip made of silicon and having multiple microwells, each microwell being used to store a single specimen organic cell and to recover the stored single specimen organic cell therefrom, wherein each microwell is of a size and shape holding just one organic cell, wherein the interior surface of said microwells is coated with a fluorocarbon film, so that the interior surface of said microwells prevents adhesion of the organic cell and facilitates recovery of the stored organic cell from the microwell.

6. I have reviewed the Office Action dated March 11, 2010, and the Advisory Action dated May 17, 2010, in which the claimed invention was rejected as obvious over U.S. Publication No. 2002/0072116 to Bhatia *et al.*, ("Bhatia"), in view of U.S. Patent No. 6,197,575 to Griffith *et al.*, ("Griffith"). I have read and understand these references.

7. It is my opinion that the claimed invention is not rendered obvious by the cited references, either when considered alone, or in combination. As evidence of my opinion, the experiments discussed below and in Embodiment 4 of the originally filed application, demonstrate that a coating of fluorocarbon film on the interior surface of the microwells greatly facilitates recovery of stored organic cells. This increase in cell recovery could not have been expected by an ordinary artisan at the time of the invention.

## 8. Experiments

Embodiment 4 of the originally filed application describes a microwell array chip, wherein the interior surfaces of the microwells are coated with a fluorocarbon film, *see* pages 32-34 of the originally filed application. Embodiment 4, which is described below, demonstrates that a greater amount of cells are recovered from microwells coated with a fluorocarbon film in comparison to microwells, which are not coated with a fluorocarbon film.

*8a. Manufacturing a microwell array chip employing a silicon substrate having fluorocarbon film on the interior surfaces of the microwells*

Fig. 12 in the originally filed application shows the steps of manufacturing a microwell array chip employing a silicon substrate. (1) Novolak resin-based positive photoresist OFPR-800, (22c), made by Tokyo Ohka Kogyo (K.K.), for example, is coated on a silicon substrate, 22b, having a silicon oxide film, 22a, and a microwell pattern, 22d, is formed. At this time, heat treatment following development is conducted at a lower temperature than usual, (from about 100 to 110°C). (2) Silicon substrate, 22b, is etched by introducing a silicon etching gas such as SF6 into a plasma dry etching device to form microwells, 22e. (3) A CxFy-based gas is introduced into the same etching device to conduct plasma film formation. At this point, a fluorocarbon film, 22f, forms inside the wells and on the silicon substrate surface. This step can also be conducted by conveying the substrate into a plasma CVD device and conducting the same processing. (4) The substrate removed from the device is immersed in an organic solvent such as methanol or acetone to remove the photoresist. At this time, the fluorocarbon film formed on the resist is also lifted off. (5) A microwell array chip is obtained in which the outermost surface of the silicon substrate is coated with a silicon oxide film, 22a, and the interior of the wells is coated with an inert fluorocarbon film, 22f.

Fig. 11 in the originally filed application depicts a schematic drawing of the microwell array chip. As is evident from the embodiment depicted in Fig. 11, multiple microwell patterns, 21b, are arranged on the surface of silicon substrate, 21a. The size of each microwell, 21b, is from several micrometers to several tens of micrometers. As noted above, a fluorocarbon film

was formed with a CxFy-based gas on the sidewall of each well formed, and a surface energy reducing effect created an inert state, 21c. Fluorocarbon film, 21c, which exhibited a hydrophobic property, was selectively formed within the microwells, but was not present on the outermost surface, 21d, of the silicon. The organic cell entering microwell, 21b, tended not to readily adhere. The effect of providing a fluorocarbon film inside the microwells was particularly marked when the microwell was deep.

*8b. Determining the array (fill) rate of a microwell array chip*

The array (fill) rate of a microwell array chip, obtained by the above-described method, was evaluated by the same method as in Embodiment 3 of the originally filed application. Briefly, $10^5$ cells/microliter were placed in Hank's balanced salt solution (HBSS) for storage. Each of the cells was fluorescently stained with CELLTRACKER™ Orange, which emitted fluorescence at the excitation wavelength, (532 nm), of the fluorescence scanner employed in measurement. The stained cells were planted on the silicon chip with a micropipette. The planting was repeated three times and cells that had not entered wells were washed off. The chip was covered with a glass slide to prevent drying out, and the fluorescent intensity was read with a microarray scanner. A total of 4,500 wells on the chip were selected and the number of wells emitting fluorescence was counted. The array rate (fill rate) was calculated as: Array rate (fill rate) = (number of wells emitting fluorescence/4,500) x 100.

*8c. Determining the collection rate of cells*

The collection rate, that is, the number of wells from which cells were successfully removed)/number of wells randomly selected) x 100, was evaluated by the following method. 1) Based on the above-described cell array (fill) rate evaluation method, cells were planted in a microwell array. 2) A random number of wells, (about 10 to 30), were selected and the cells were removed from the wells with a micromanipulator. At that time, the removal was not conducted aggressively; caution was exercised to employ the same removal conditions for each well. 3) For the randomly selected number of wells, the ratio of the number of wells from which cells were successfully removed was denoted as the collection rate.

## 9. Results

Table 2 in the originally filed application, which is also depicted below, describes the evaluation results for a microwell array chip in which a fluorocarbon film was formed within the wells in comparison to the results for a microwell array chip without fluorocarbon film. The same well diameter and depth were selected for all of the samples. As noted above, the concentration of the planted cells was $10^5$ cells/microliter.

Table 2

| Sample | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| Array (fill) rate | 99.4% | 99.2% | 99.2% | 99.4% | 99.3% | 98.9% |
| Collection rate | 0% | 10% | 6.7% | 50% | 30% | 89.3% |

Sample specifications: Well diameter: 11 micrometers, depth 30 micrometers

Samples A-C: No coating, etching time: 8 minutes

Samples D-F: Coating present, etching time: 8 minutes + coating time: 1 minute

As described in Table 2, the collection rates of samples A to C, which do not have coating on the interior surface of microwells, ranges from only 0% to 10%. In contrast, the collection rate of samples D to F, which have a coating of fluorocarbon film on the interior surface of microwells, ranges from 30% to 89.3%. The high collection rates of samples D to F, demonstrate that the presence of the fluorocarbon film facilitates recovery of the stored organic cell from the microwells.

10. It is my opinion that an ordinary artisan could not have expected from the cited references that fluorocarbon coating of the microwells would have resulted in the above-described high cell collection rates. Bahtia does not describe fluorocarbon films. Further, Griffith merely indicates that fluorocarbon film is useful for the adsorption of extra-cellular matrix proteins, which enable cell adhesion to the walls of the channels. Accordingly, an ordinary artisan could not have reasonably expected from either of these references, when considered alone or in combination, that fluorocarbon coating could have significantly enhanced the collection rates of cells from microwells.

11. In view of the foregoing, it is my opinion that the claimed invention is not obvious in view of the Bhatia and Griffith references.

**STATEMENT UNDER 18 U.S.C. § 1001**

I hereby declare that all statements made herein of my own knowledge are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

By: Tsutomu Obata Date: 08/10/10

Dr. Tsutomu Obata